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PRINCIPAL INVESTIGATOR: Nicolas G. Azios
Surangani Dharawardhane, Ph.D.

CONTRACTING ORGANIZATION: University of Texas at Austin
Austin, Texas 78712-1500

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Nicolas G. Azios
Surangani Dharawardhane, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Texas at Austin
Austin, Texas 78712-1500

E-Mail: nazios@mail.utexas.edu

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13. ABSTRACT (Maximum 200 Words)
The purpose of the proposed study is to determine rapid effects of resveratrol, genistein, and daidzein on Rho GTPases and downstream effectors involved in breast cancer cell motility. The scope of the study is to determine signaling pathways affected by the phytoestrogens regarding cell motility and to use confocal microscopy to track *in vivo* the metastatic potential of cells treated with phytoestrogens in mice. To date, there are no major findings associated with this investigation because at the same time the PI was awarded this DOD fellowship, the PI was also awarded a NIH fellowship and chose to take the NIH award for research support. Dr. Katherine Moore advised the PI to keep the DOD award and transfer the support to a new graduate student in fall 2005. The PI has not initiated the proposed project nor has the PI used any of the funding. In September 2005, a new fellow will be named and the funding will be transferred (please see Appendix 1 for e-mail correspondence between Judy Pawlus and the PI).
No key accomplishments or reportable outcomes have been completed as the project was never initiated and has been put on hold until a new recipient is named.

14. SUBJECT TERMS
Resveratrol, genistein, daidzein, phytoestrogens, Rho GTPases

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Introduction:

Estrogen (17-beta-estradiol; E2) is a human female steroid hormone that plays critical roles in normal breast development as well as breast cancer formation. But its effects on breast cancer progression are not well characterized. Non-genomic effects of E2 have been reported from a variety of cell types including breast cancer cells (1). These rapid effects may be significant in signaling to the actin cytoskeleton as well as proteins involved in cancer cell motility and migration.

Phytoestrogens are naturally occurring estrogen-like plant compounds which are known to act as agonists or antagonists of E2 and may have protective action against some cancers and prevent the undesirable symptoms of menopause (2). Most studies on phytoestrogen action in breast cancer have focused on their relevance in prevention of the onset of breast cancer, though some studies show that phytoestrogens also have effects on metastasis.

Genistein and daidzein (phytoestrogens from soy) inhibited melanoma metastasis, intestinal adenocarcinoma, and prostrate carcinoma metastasis in mouse and rat models (3). However, most of these studies monitored long-term exposure of genistein and thus its effect on down-regulation of genes involved in invasion and angiogenesis (4). Other studies actually demonstrate increased tumor growth and lung metastasis upon administration of soybean extracts (5). The *in vivo* actions of genistein may therefore extend beyond those traditionally implicated in chemoprevention like antiproliferation. Thus, genistein may act *in vivo* by blocking additional stages of breast cancer progression such as those stages resulting in invasion and metastasis (6).

Resveratrol (a phytoestrogen from grapes/red wine) has been demonstrated to reduce hepatoma cell invasion and metastasis *in vitro* as well as in mice with hepatoma and Lewis lung carcinoma tumors (7). Recently, resveratrol was shown to affect actin cytoskeletal organization and inhibit invasion of ERαβ (+) primary human breast cancer cell line MCF-7 (8) and in ERα (-) β (+) MDA-MB-231 (9)

The members of Rho family of small GTPases are examples of potential key regulators of the initial steps of cancer metastasis. Rho GTPases are directly implicated in the regulation of the actin cytoskeleton and focal adhesion turnover during directed cell motility of invading cancer cells and contribute to metastasis (10).

Body:

The purpose of the proposed study is to determine rapid effects of resveratrol, genistein, and daidzein on Rho GTPases and downstream effectors involved in breast cancer cell motility. The scope of the study is to determine signaling pathways affected by the phytoestrogens regarding cell motility and to use confocal microscopy to track *in vivo* the metastatic potential of cells treated with phytoestrogens in mice.

To date, there are no major findings associated with this investigation because at the same time the PI was awarded this DOD fellowship, the PI was also awarded a NIH fellowship and chose to take the NIH award for research support. Dr. Katherine Moore advised the PI to keep the DOD award and transfer the support to a new graduate student in fall 2005. The PI has not initiated the proposed project nor has the PI used any of the funding. In September 2005, a new fellow will be named and the funding will be transferred (please see Appendix 1 for e-mail correspondence between Judy Pawlus and the PI).

Key research accomplishments:
No key accomplishments have been completed as the project was never initiated and has been put on hold until a new recipient is named.

Reportable outcomes:
No reportable outcomes have been completed as the project was never initiated and has been put on hold until a new recipient is named.

Conclusions:
To date, there are no conclusions because the project was never initiated and has been put on hold until a new recipient is named.

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1. Nicholson RI, Hutcheson IR, Harper ME, Knowlden JM, Barrow D, McClelland RA et al. Modulation of epidermal growth factor receptor in endocrine-resistant, estrogen-receptor-positive breast cancer. *Ann N Y Acad Sci JID - 7506858* 2002;963:104-15.

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Appendix 1: E-mail correspondence between Dr. Katherine Moore, Judy Pawlus, and the PI

Date: Fri, 11 Feb 2005 06:59:08 -0500

From: "Pawlus, Judy K Ms USAMRMC" <judy.pawlus@us.army.mil>

To: nazios@mail.utexas.edu

Subject: RE: annual summary report W81XWH-04-1-0381

You should write a report indicating in the body that there has been no progress to date and the reason why.

-----Original Message-----
From: nazios@mail.utexas.edu [mailto:nazios@mail.utexas.edu]
Sent: Thursday, February 10, 2005 2:19 PM
To: Pawlus, Judy K Ms USAMRMC
Subject: Re: annual summary report W81XWH-04-1-0381

Ms. Pawlus,
This e-mail is regarding my annual summary report (W81XWH-04-1-0381) for my DOD predoctoral fellowship (BC031891). I have been in contact with Dr. Katherine Moore of Grants Management CDMRP (see below e-mail communications) concerning my particular situation. At the same time I was awarded a DOD fellowship, I was also awarded a NIH fellowship and chose to take the NIH for support. Dr.
Moore said we could hold onto the DOD award and transfer the supprt to a new graduate student in fall 2005. I have not worked the the project proposed for the DOD fellowship nor have a spent any of the funding money. There is no annual report to write. In fall 2005 a new fellow will be named and the funding will be transfered to them. Please tell me how to proceed with the annual report.
Thank you for your time.
Nicolas Azios
Ph.D. candidate/Plant Biology Graduate Program University of Texas at Austin Molecular Cell and Developmental Biology Dept.
(512) 475-8129
nazios@mail.utexas.edu

e-mail communications with Dr. Moore
Dear Nicolas,
Congratulations on receiving an NIH fellowship! As was stated in an earlier message from Karen Stoller, the DOD award cannot be converted to supplies only. The award is meant to support a student during their research phase of their degree program. At this point, I think that the best option would be for your mentor to nominate a new student to the fellowship. The eligibility criteria for the new fellow would be the same as when you applied for the award. Also, the research project can be changed to fit the new fellows interest, but must remain relevant to breast cancer. However, your University does have the option of returning the grant at any time.
I apologize for the delay in replying to your question, but please feel free to contact me at any time.

Sincerely,
Katherine Moore, Ph.D.

Katherine Moore, Ph.D.
Grants Management
CDMRP
phone: 301-619-6882
FAX: 301-619-7796

Dear Dr Dharmawardhane,
It sounds as Nicolas has not done any work on the project, so I believe that an abstract is not necessary, and he does not need to attend the meeting. We will need to extend the performance period of the grant so the new PI will have the full 3 years to complete the training. I suggest that your new student be required to attend the next Era of Hope meeting. When you have identified a new student for the fellowship,

please let me know, and I will help with transferring the fellowship on our end.

Sincerely,
Kathy Moore
Katherine Moore, Ph.D.
Grants Management
CDMRP
phone: 301-619-6882
FAX: 301-619-7796

Quoting "Pawlus, Judy K Ms USAMRMC" <judy.pawlus@us.army.mil>:

> We realize how busy our researchers are at this time of year.
> However, see the attached letter. Your organization entered into an
> agreement with the Army in which there are reporting obligations now
> due. As Principal Investigator it is important to our Army programs
> that you meet this suspense. An annual summary report is due to this
Command not
> later than March 25, 2005. Thank you for your attention to this
> matter. We look forward to receipt of this report not later than
this
> suspense.